

Anti-Parasitic Activity of ssDNA-Aptamers Targeting the Poly(A)-Binding Protein in *Leishmania donovani* Promastigotes

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Abstract:

Background: Visceral leishmaniasis caused by *Leishmania donovani* still depends on a small group of drugs whose value is diminished by toxicity, cost and resistance, so targets specific to the parasite remain attractive. The cytoplasmic poly(A)-binding protein (PABP) of *Leishmania* governs mRNA stability and translation, processes on which these parasites lean heavily because they lack conventional transcriptional control.

Aim: To evaluate the antiparasitic activity against *L. donovani* promastigotes of two distinct PABP-targeting ssDNA aptamers complexed with Lipofectamine 2000, AP-7/LF and AP-11/LF.

Methods: Growth inhibition was measured by the MTT assay at 24 and 48 hours, and PABP protein abundance was quantified by Western blot densitometry against a GAPDH loading control.

Results: Both complexes inhibited promastigote growth in a concentration- and time-dependent manner, but AP-11/LF was the stronger agent throughout: its IC₅₀ values were 234 nM after 24 hours and 194 nM after 48 hours against 1810 nM and 1300 nM for AP-7/LF, a difference of roughly 7.7-fold and 6.7-fold, respectively. The Western blot results followed the same ranking, as AP-11/LF lowered PABP to 0.51 of the untreated control (a 48.6% reduction, $p = 0.001$), whereas AP-7/LF lowered it to 0.67 (a 32.9% reduction, $p = 0.018$). The lipofectamine alone and the carrier-free aptamers produced only small, non-significant changes.



Conclusion: The poly(A)-competitive aptamer (AP-11) outperformed the high-affinity, non-competitive aptamer (AP-7) at both the growth and the protein level, supporting PABP disruption as a workable starting point for antileishmanial drug development.

Keywords: Poly(A)-binding protein; ssDNA aptamers; Visceral leishmaniasis; promastigotes; Lipofectamine 2000.

1. Introduction

Leishmaniasis, a vector-borne disease caused by protozoan parasites of the genus *Leishmania*, represents one of the most significant yet neglected tropical diseases (NTDs) impacting global public health¹. Its visceral form, also called kala-azar and caused chiefly by *L. donovani*, is fatal if untreated, and the World Health Organization continues to list it among the priority neglected diseases of tropical and sub-tropical regions^{2,3}. Yet, current treatment of leishmaniasis still relies on a limited group of drugs. Although these drugs have saved lives for a long period, their use is limited because of their toxicity, high cost, parenteral administration, long treatment courses, regional differences in efficacy and the emergence of resistant parasites^{4,5}. Additionally, no licensed vaccine is currently available for routine prevention of human leishmaniasis, which strengthens the need for new therapeutic strategies with novel mechanism of action^{5,6}.

Trypanosomatids handle gene expression in an unusual way, and that biology points to one such target. Protein-coding genes are arranged in long polycistronic arrays transcribed constitutively, individual messages are released by trans-splicing and polyadenylation, and promoter-driven control of single genes is essentially absent^{7,8}. The parasite therefore tunes protein levels after transcription, through mRNA stability and translation, rather than at the point of initiation⁹. The poly(A)-binding protein sits at the center of that system. It coats the 3' poly(A) tail of mRNA, links the tail to the 5' cap-binding complex through

eIF4G partners, and so couples message stability to translation¹⁰⁻¹².

In *Leishmania*, the picture is richer than in mammalian cells, which carry one dominant cytoplasmic PABP: the parasite expresses several PABP homologues with partly separated sets of bound transcripts, and PABP1 is the homologue tied most closely to the main cap-dependent initiation complex^{13,14}. Removing or disabling PABP would be expected to disturb translation across a wide range of the transcriptome rather than block a single pathway, because the protein occupies a vital position in the regulatory network^{15,16}. Two features make it a workable drug target. Leishmanial PABP shares only about 35-40% sequence identity with human PABPC1, which leaves a window for ligands that bind the parasite protein while sparing the host one^{17,18}.

Aptamers are a natural fit for that opportunity. They are short single-stranded DNA or RNA oligonucleotides that fold into defined three-dimensional shapes and bind their targets with antibody-like affinity and specificity¹⁹. They are isolated *in vitro* by the iterative selection method SELEX²⁰. Against antibodies, they offer chemical synthesis, easy modification, thermal stability, low immunogenicity and low production cost^{21,22}. Two aptamers have reached the clinic, pegaptanib for neovascular age-related macular degeneration and avacincaptad pegol for geographic atrophy, which establishes the class as a real therapeutic format rather than a laboratory curiosity^{23,24}. In parasitology, however, most aptamer work has stopped at detection and molecular recognition: aptamers have been raised against protozoan antigens and used mainly for



diagnosis, with small progress towards direct antiparasitic activity²⁵. Against *Leishmania* specifically, the most sustained programme has produced ssDNA aptamers against KMP-11, histone H2A and histone H3, all developed as diagnostic reagents^{26–29}. The same programme also produced PABP-binding aptamers, and these are the subject of the present work.

Guerra-Pérez *et al.* (2015)³⁰ used 12 rounds of SELEX against recombinant *L. infantum* PABP and isolated three high-affinity ssDNA aptamers, of which two are studied in this study: Aptamer-7 (AP-7), 40 nucleotides (nt), 42.5% GC, and dissociation constant (K^d) is 6.0 ± 2.6 nM, and Aptamer-11 (AP-11), 40 nt, 80% GC, and K^d is 10.8 ± 2.7 nM. In poly(A)-competition assays, AP-11 reduced the binding of PABP to poly(A) RNA, whereas AP-7 bound the protein tightly but did not measurably disturb that interaction³⁰. AP-7 therefore recognizes PABP without blocking its central activity, while AP-11 interferes with the poly(A) binding itself. Until now, this distinction had only been shown biochemically, with purified protein. Whether it carries over into living parasites was unknown, because no aptamer had been tested for direct antileishmanial activity against intact *Leishmania* cells.

The present study addresses this question in extracellular promastigotes. Both aptamers were complexed with the cationic lipid carrier Lipofectamine 2000 (LF) to carry the negatively charged oligonucleotides across the parasite membrane, and the complexes were assessed in two complementary ways: growth inhibition by the MTT assay over 24 and 48 hours, and PABP protein expression by Western blot.

2. Materials and Methods

2.1 Parasite isolate and promastigote culture

The *L. donovani* isolate MHOM/IQ/2005/MRU15 was used throughout; species identity had been

confirmed previously by PCR genotyping³¹. Promastigotes were grown in RPMI-1640 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HIFBS) and penicillin-streptomycin (100 U/mL and 100 µg/mL). Cultures were kept in tissue-culture flasks at 26 °C and passaged routinely to hold the population in logarithmic growth. Late-stationary parasites were obtained by allowing growth to continue for eight days at 26 °C. Density was read on an automated dual-chamber counter. Staging was checked using an inverted microscope at 20× and 40×, where acceptable promastigote cultures showed elongated, flagellated cells with the rosette clustering typical of late-log or stationary phase.

2.2 Aptamers and complex formation

The two ssDNA aptamers were obtained as lyophilized powders (Macrogen, South Korea). The sequences were AP-7, 5'-CCAGAGTGAACACAAAGAAATTGA AATACGTAAGCTCCGG-3', and AP-11, 5'-GGCCCCCTCCTCCCTCCCCACCCG AACTATTCCCCCCC-3'. Each was reconstituted to 100 µM in nuclease-free water and stored at -20°C as single-use aliquots. Before each assay, stock was diluted in HEPES folding buffer to the required working concentration, heated to 95°C for 5 minutes in a dry heat block to promote intramolecular folding, and then left to cool to room temperature (25°C) before use. Complexes with Lipofectamine 2000 (LF) (Invitrogen/Life Technologies) were assembled following the manufacturer's instructions: LF reagent (1.5 µL in 125 µL Opti-MEM) was held for five minutes at 25°C, combined with folded aptamer prepared at twice the target treatment concentration, mixed gently, and left for twenty minutes at room temperature so that the cationic carrier could self-assemble around the oligonucleotide.



2.3 Experimental groups

Each assay carried matched control arms so that any effect could be attributed to the intact complex rather than to either component. The arms were the aptamer-LF complex (AP-7/LF or AP-11/LF); an untreated negative control of promastigotes culture only; sodium stibogluconate as a positive control; the free aptamer without carrier (FAP-7 or FAP-11) at the highest aptamer dose tested; and the carrier LF alone (diluted Lipofectamine 2000 without aptamer). The free-aptamer and vehicle-only arms exist to separate the contribution of the delivered aptamer from that of the carrier or the naked oligonucleotide.

2.4 Growth inhibition by the MTT assay

Anti-promastigote activity was measured with a tetrazolium (MTT) reduction assay,

a colorimetric readout of viable-cell metabolic activity^{32,33}. Late-stationary promastigotes were seeded into flat-bottom 96-well plates at 1×10^6 cells/mL (100 μ L per well in RPMI-1640 with 10% HIFBS). Two-fold dilution series were applied, spanning 78.125-10,000 nM for AP-7/LF and 12.5-1,600 nM for AP-11/LF, with 20 μ L of each dilution dispensed into the appropriate wells and all five arms present on every plate. After 24 or 48 hr. at 26°C, MTT was added to 0.5 mg/mL and the plates were incubated for a further 4 hours, so that viable cells could form insoluble formazan. The supernatant was aspirated, the formazan dissolved in DMSO (0.5% v/v final) with 5 minutes of shaking, and absorbance read at 570 nm against a 630 nm reference on a Bio-Rad plate reader. Cell viability was calculated as:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated well} - \text{Absorbance of blank}}{\text{Absorbance of untreated control} - \text{Absorbance of blank}} \times 100^{33}$$

and growth inhibition percentage (GI %) as: $\text{GI \%} = 100 - \text{Viability \%}$ ³³

Each concentration was tested in triplicate within a run, across three independent runs ($n = 3$). IC_{50} values were derived by fitting a four-parameter logistic (variable-slope) curve to the log-concentration versus inhibition data in GraphPad Prism (version 8.0.2 Software, USA).

2.5 Protein extraction and Western blot

Treated and control promastigotes were harvested, washed three times in ice-cold PBS (pH 7.4) and lysed in freshly reconstituted RIPA buffer, with cell rupture completed by ice-cooled water-bath sonication. Debris was cleared by centrifugation at 12,000g (10 min, 4°C) and total protein in the supernatant measured colorimetrically by the BCA method at 562 nm against a BSA standard series³⁴. Equal protein loads were denatured in $5 \times$ SDS loading buffer at 95°C for 5 minutes, resolved on 4-12% precast gel (12-well format; NZY-Tech®, Portugal), and

transferred to 0.45 μ m polyvinylidene fluoride (PVDF) membranes. Membranes were blocked for 90 minutes in TBST with 5% skimmed milk, incubated overnight at 4°C with a rabbit anti-PABPC1 primary antibody (1:1,000 in 5% milk-TBST), washed, and incubated for 60 minutes with an HRP-conjugated anti-rabbit secondary antibody. Protein separation was performed with a pre-stained molecular-weight ladder, and the PABP-associated immunoreactive band was evaluated at the expected apparent molecular mass of approximately 65 kDa. Bands were developed by enhanced chemiluminescence and imaged on a Bio-Rad ChemiDoc XRS+. Band intensities for PABP and the GAPDH loading control were measured by ImageJ densitometry; normalized PABP expression was the PABP/GAPDH ratio expressed as a fold-change against the untreated control, which was set to 1.0. Four arms were run on each blot: untreated, LF alone, free aptamer



(FAP-7 or FAP-11), and the complete complex (AP-7/LF or AP-11/LF).

2.6 Statistical Analysis

Statistical analyses were carried out in GraphPad Prism, version 8.0.2 Software (USA). Quantitative data were expressed as the mean $\pm$ standard error of the mean (SEM). Growth inhibition experiments were performed in triplicate per condition per independent assay, and the results of three independent assays ($n = 3$) were used for statistical analysis. Nonlinear regression analysis of the dose-response curves was used to determine the IC_{50} values using a four-parameter logistic (4PL) variable-slope model. For nonlinear regression analyses, 95% confidence intervals for IC_{50} estimates were calculated using the profile-likelihood method, and goodness of fit was assessed using the coefficient of determination (R^2) and residual standard deviation (Sy.x). Plotting of the dose-

response curves was performed using GraphPad Prism. For Western blot data densitometry, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparisons test (all pairwise comparisons) was used in comparisons of the groups.

3. Results

3.1 Growth inhibition of promastigotes

3.1.1 The Effect of Aptamer-7/Lipofectamine Complex

AP-7/LF showed a concentration-dependent inhibitory effect against *L. donovani* promastigotes growth following 24 and 48 hr. times of incubation. After 24 hr., growth inhibition ranged from $4.33 \pm 1.69\%$ at 78.125 nM to $71.06 \pm 1.72\%$ at 10000 nM, producing an IC_{50} value of 1810 nM (Figure 1, A). After 48 hours of incubation, growth inhibition ranged from $4.70 \pm 1.33\%$ to $64.14 \pm 1.16\%$, with an IC_{50} of 1300 nM (Figure 1, B).

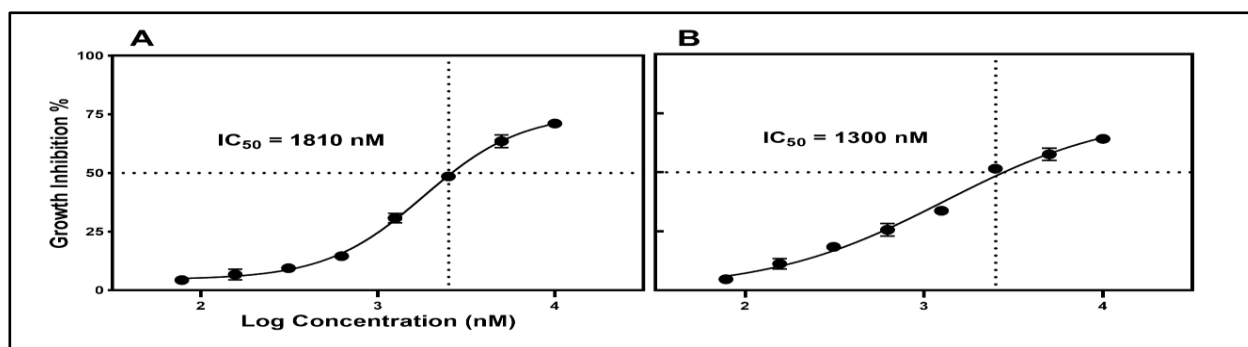


Figure (1): Dose-response inhibition curves of *L. donovani* promastigotes treated with AP-7/LF for 24 hr. (A) and 48 hr. (B). Promastigotes were incubated with two-fold serial dilutions of AP-7/LF (78.125-10,000 nM), and growth inhibition (%) was determined by MTT assay. Curves were fitted by nonlinear regression using a 4PL variable slope model. IC_{50} values were 1810 nM at 24 hr. (A) and 1300 nM at 48 hr. (B), indicated by dotted lines. The 4PL fits yielded $R^2 = 0.9893$ at 24 hr. and $R^2 = 0.9764$ at 48 hr. Data represent mean $\pm$ SEM ($n = 3$).

3.1.2 The Effect of Aptamer-11/Lipofectamine Complex

AP-11/LF showed a concentration-dependent inhibitory effect against *L. donovani* promastigotes following both times of incubation. After 24 hr., growth inhibition ranged from $5.9 \pm 1.69\%$ at 12.5

nM to $68.23 \pm 0.63\%$ at 1600 nM, producing an IC_{50} of 234 nM (Figure 2, A). At 48 hr. of incubation, growth inhibition ranged from $7.07 \pm 1.11\%$ to $71.43 \pm 2.14\%$, with an IC_{50} of 194 nM (Figure 2, B).



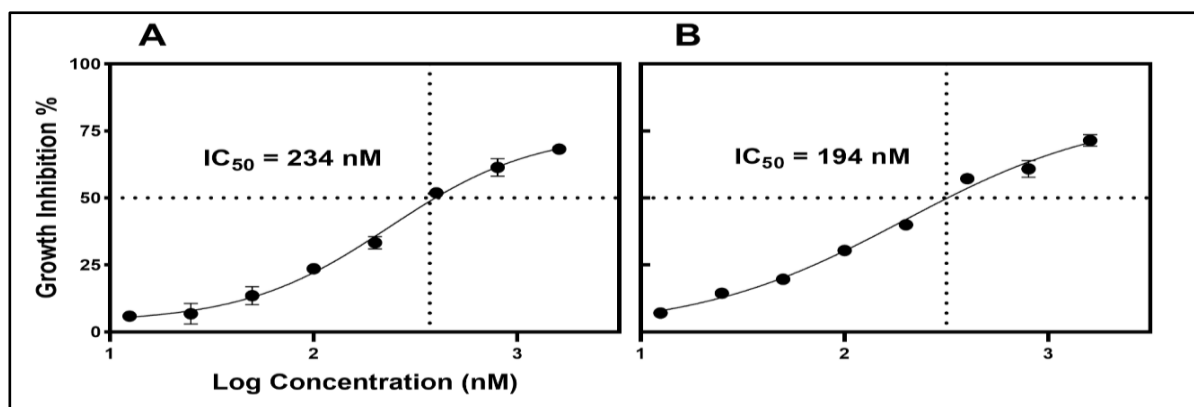


Figure (2): Dose-response inhibition curves of *L. donovani* promastigotes treated with AP-11/LF for 24 hr. (A) and 48 hr. (B). Promastigotes were incubated with two-fold serial dilutions of AP-11/LF (12.5–1600 nM) and growth inhibition (%) was determined by MTT assay. Curves were fitted by nonlinear regression using a 4PL variable slope model. IC_{50} values were 234 nM at 24 hr. (A) and 194 nM at 48 hr. (B), indicated by dotted lines. Data represent mean $\pm$ SEM (n = 3).

3.1.3 Comparative Analysis of Anti-Promastigote Activity

A direct comparison of the anti-promastigote potencies of the two aptamer complexes revealed that AP-11/LF was consistently and substantially more effective than AP-7/LF when inhibiting *L. donovani* promastigotes growth at both time points (Table 1). After 24 hr., the IC_{50} of AP-11/LF (234 nM) was about 7.7-fold lower than that of AP-7/LF (1810 nM). After 48 hr., this difference remained pronounced at 6.7-fold (194 nM vs. 1300

nM). Both complexes exhibited time-dependent enhancement of activity, with lower IC_{50} values after 48 hr. compared to 24 hr., however, the degree of time-dependent improvement differed between the two aptamers. The IC_{50} of AP-7/LF decreased from 1810 nM after 24 hr. to 1300 nM after 48 hr., representing 1.4-fold improvement in the aptamer activity; whereas AP-11/LF showed a more modest 1.2-fold improvement in activity as the IC_{50} decreased only from 234 to 194 nM.

Table (1): Comparative IC_{50} values (nM) and fold-differences for AP-7/LF and AP-11/LF against *L. donovani* promastigotes.

Aptamer Complex	IC_{50} (nM) (95% CI)		Potency fold difference 24 - 48 hr.
	24 hr.	48 hr.	
AP-7/LF	1810 (1557–2193)	1300 (858.1–5872)	1.4-fold
AP-11/LF	234 (176.2–379.3)	194 (134.8–562.0)	1.2-fold
AP-7/AP-11 IC_{50} ratio*	7.7-fold	6.7-fold	-

* IC_{50} ratio = IC_{50} (AP-7/LF) $\div$ IC_{50} (AP-11/LF); The 95% confidence intervals were obtained by profile-likelihood nonlinear regression using a four-parameter logistic model.



3.2. PABP protein expression in promastigotes

3.2.1 The effect of Aptamer-7/Lipofectamine Complex

Western blot analysis of *L. donovani* promastigotes treated with AP-7/LF exhibited a modest reduction in PABP protein expression compared with untreated controls (Figure 3, A). Densitometric quantification, normalized to the GAPDH loading control, showed that PABP expression was reduced to 0.671 ± 0.080 in

the AP-7/LF-treated group, corresponding to about 32.9% reduction compared to the control (Figure 3, B). Tukey's multiple comparisons test identified the Control vs. AP-7/LF comparison as the only pairwise difference reaching statistical significance ($p = 0.018$). In contrast, lipofectamine alone (LF) and the free aptamer alone (FAP-7) produced a statistically non-significant reduction in PABP expression compared to the untreated control ($p = 0.419$ and 0.358 , respectively), (Table 2).

Table (2): The effect of LF, FAP-7 and AP-7/LF on PABP expression in *L. donovani* promastigotes quantified by western blot densitometry.

Group	Mean $\pm$ SEM	<i>p</i> -value vs. Control	Reduction % vs. Control
Control	1.00	-	-
LF	0.89 ± 0.01	0.419	10.3
FAP-7	0.88 ± 0.02	0.358	11.3
AP-7/LF	0.67 ± 0.08	0.018	32.9

PABP: poly(A)-binding protein; LF: Lipofectamine 2000; FAP-7: free AP-7 without Lipofectamine 2000; AP-7/LF: AP-7 complexed with Lipofectamine 2000; SEM: standard error of the mean.

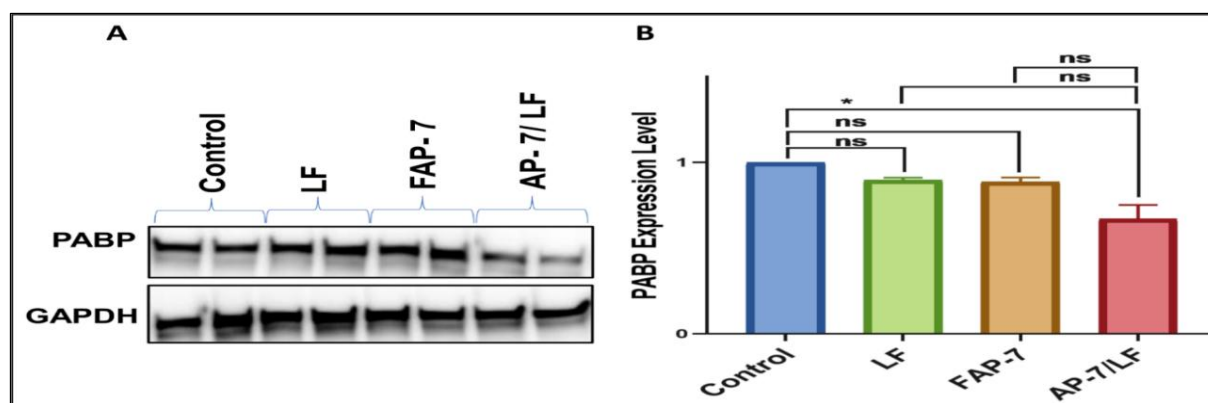


Figure (3): Western blot analysis of PABP expression in *L. donovani* promastigotes. (A) Representative PABP-associated band at the expected apparent molecular mass of approximately 65 kDa, together with GAPDH loading-control bands, in untreated control, LF, FAP-7 and AP-7/LF groups; (B) Densitometric quantification of PABP band intensity expressed compared to the untreated control (set to 1). The data are displayed as mean $\pm$ SEM ($n = 3$). Tukey's multiple comparisons test was used after standard one-way ANOVA for statistical analysis. ns = not significant; * $p < 0.05$.

3.2.2 The Effect of Aptamer-11/Lipofectamine Complex

The results of the Western blot regarding the expression of PABP by *L. donovani* promastigotes treated with AP-11/LF showed a pronounced reduction in this protein expression compared to untreated controls (Figure 4, A). Densitometric quantification showed that PABP expression in the AP-11/LF-treated group

was reduced to 0.514 ± 0.047 , corresponding to about 48.6% reduction compared to the control (Table 3). Tukey's multiple comparisons test following one-way ANOVA confirmed a significant reduction in PABP expression in the AP-11/LF group compared with the untreated control ($p = 0.001$), (Figure 4, B).

Table (3): The Effect of LF, FAP-11 and AP-11/LF on PABP expression in *L. donovani* promastigotes quantified by western blot densitometry.

Group	Mean $\pm$ SEM	<i>p</i> -value vs. Control	Reduction % vs. Control
Control	1.00	-	-
LF	0.882 ± 0.039	0.1	11.77
FAP-11	0.896 ± 0.020	0.2	10.38
AP-11/LF	0.514 ± 0.047	0.001	48.64

PABP: poly(A)-binding protein; LF: Lipofectamine 2000; FAP-11: free AP-11 without Lipofectamine 2000; AP-11/LF: AP-11 complexed with Lipofectamine 2000; SEM: standard error of the mean.

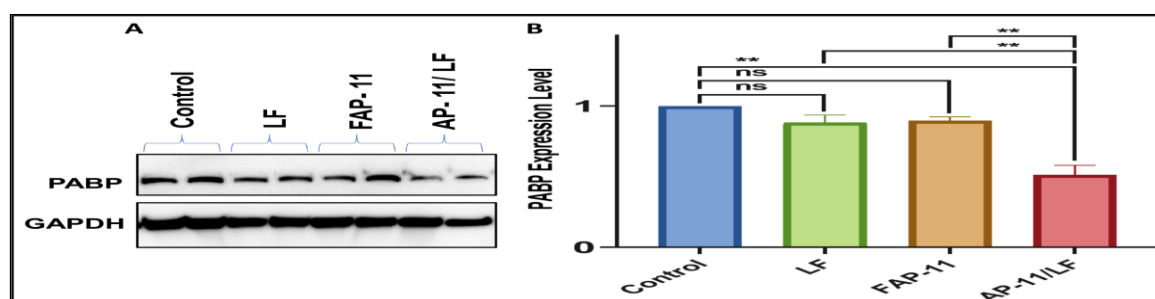


Figure (4): Western blot analysis of PABP expression in *L. donovani* promastigotes (A) Representative PABP-associated band at the expected apparent molecular mass of approximately 65 kDa, together with GAPDH loading-control bands, in untreated control, LF, FAP-11 and AP-11/LF groups; (B) Densitometric quantification of PABP band intensity compared to untreated control (set to 1). Data are displayed as mean $\pm$ SEM ($n=3$). Tukey's multiple comparisons test was used after standard one-way ANOVA for statistical analysis. ns = not significant; ** $p < 0.01$.

3.2.3 Comparative Effect of AP-7/LF vs AP-11/LF on PABP Expression

Comparative densitometric analysis revealed that both aptamers' complexes significantly reduced the expression of

PABP compared to the untreated control. However, AP-11/LF produced a greater reduction (48.6%) than AP-7/LF (32.9%) (Table 4).

Table (4): Comparative Summary of AP-7/LF vs AP-11/LF on PABP Expression in Promastigotes.

	AP-7/LF	AP-11/LF
Mean $\pm$ SEM	0.671 $\pm$ 0.080	0.514 $\pm$ 0.047
<i>p</i> -value	0.018	0.001
Reduction %	32.9%	48.6%

AP-7/LF: AP-7 complexed with Lipofectamine 2000; AP-11/LF: AP-11 complexed with Lipofectamine 2000; SEM: standard error of the mean.

4. Discussion

The present study shows that the PABP-targeting aptamers AP-7 and AP-11 inhibit the growth of *Leishmania donovani* promastigotes when complexed with Lipofectamine 2000, with AP-11/LF showing consistently greater activity. AP-11/LF produced IC₅₀ values of 234 nM at 24 hr. and 194 nM at 48 hr., compared with 1810 and 1300 nM for AP-7/LF, corresponding to approximately 7.7- and 6.7-fold greater potency, respectively. Western blot analysis showed the same relative pattern: AP-11/LF reduced PABP abundance by 48.6% (*p* = 0.001), whereas AP-7/LF produced a 32.9% reduction (*p* = 0.018). In contrast, LF alone and the LF-free aptamers produced only minor, statistically non-significant changes.

The greater activity of AP-11 is consistent with the known functional difference between the two aptamers³⁰. AP-11 interferes with PABP binding to poly(A), whereas AP-7 binds PABP without measurably disrupting this interaction. Therefore, the stronger growth inhibition and greater PABP reduction observed with AP-11/LF support an association between interference with the PABP-poly(A) axis and enhanced antiparasitic activity^{35–37}.

However, the present study does not directly demonstrate intracellular PABP-poly(A) dissociation, impaired translation, or altered mRNA stability. Direct mechanistic approaches such as RNA immunoprecipitation, polysome profiling, and global protein-synthesis assays will be needed to establish causality.

A limitation of the present study is that its primary experiments were performed in promastigotes rather than the clinically relevant amastigote stage. This issue was subsequently investigated in a separately published complementary study³⁸, in which AP-11/LF also showed greater activity against *L. donovani* amastigotes, with IC₅₀ values of 130 and 80 nM at 24 and 48 hr. compared with 550 and 460 nM for AP-7/LF. That study also demonstrated PABP suppression in an infected RAW264.7 macrophage model. Because these findings have already been published, they are cited here for context rather than presented as new results. Importantly, direct intracellular parasite burden or intracellular IC₅₀ was not measured; therefore, the intracellular findings should be regarded as evidence of target-associated modulation rather than direct proof of parasite clearance.

Host-cell selectivity was also examined in the complementary study³⁸. AP-7/LF produced RAW264.7 CC₅₀ values of 40,000 and 21,500 nM at 24 and 48 hr., whereas AP-11/LF produced values of 8,330 and 6,725 nM. The corresponding selectivity indices were 72.7 and 46.7 for AP-7/LF and 64 and 84 for AP-11/LF. These findings support further investigation of AP-11 despite its greater macrophage cytotoxicity. Nevertheless, Lipofectamine 2000 is primarily a laboratory transfection reagent, and safer delivery systems will be required for therapeutic development³⁹. The formulation-dependent response is consistent with carrier-facilitated delivery, but aptamer uptake was not directly



quantified by fluorescence, confocal microscopy, or flow cytometry.

The stability of the aptamers will also require optimization before therapeutic development. Unmodified oligonucleotides may be susceptible to extracellular and intracellular nucleases and generally show limited spontaneous membrane permeability. Future studies should therefore include serum-stability and nuclease-resistance assays. Chemical approaches such as phosphorothioate modification and, where structurally appropriate, 2'-fluoro or 2'-O-methyl substitutions may improve nuclease resistance, while PEGylation or nanoparticle-based formulations may alter persistence and biodistribution. Such modifications must be evaluated carefully because they may affect aptamer folding, target affinity and biological activity^{40–42}. Additional limitations include the use of three independent experiments (n = 3), the absence of a complete SSG concentration-response series suitable for IC₅₀ estimation, and the lack of independent anti-PABPC1 cross-reactivity validation and full uncropped blots with visible molecular-weight markers.

Overall, AP-11/LF showed consistently greater antiparasitic activity and PABP suppression than AP-7/LF. These findings support PABP as a potential antileishmanial target and identify AP-11 as the more promising of the two aptamers. However, direct evidence of intracellular target engagement, quantitative uptake, intracellular parasite clearance, optimized delivery, and *in vivo* efficacy are required.

5. Conclusion

PABP-targeting ssDNA aptamers formulated with Lipofectamine 2000 inhibited *L. donovani* promastigote growth and reduced PABP protein expression. AP-11/LF was consistently more active than AP-7/LF, supporting an association between functional interference with the

PABP-poly(A) axis and greater antileishmanial activity. Complementary published findings in amastigote and macrophage models further support the biological relevance of AP-11/LF. Nevertheless, direct mechanistic confirmation, quantitative uptake studies, intracellular parasite-burden measurements, improved delivery systems, and *in vivo* evaluation remain necessary before therapeutic application can be considered.

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